REPORT FOR *INVITRO* SUN PROTECTION FACTOR ANALYSIS AND DETERMINATION OF PA RATING

Name of the Product	**Ultra mineral Glo 40 +**
Batch No.	**1772**
Sponsor Name	**Effezascience**
UID No.	**EV/SPF/23-24/2355**
Report Issued on	**27/12/2023**
Report Revision No.	**01**

- We hereby confirm the authenticity of the study and assurance that the data is correct and accurate to the best of our knowledge and the sample was analyzed following the principle of good laboratory practices.
- Quality check was done according to the standard operating procedure of 'Mascot Spincontrol' and the report is an exact description of all the procedure, operations and data noticed throughout the analysis.
- Mascot Spincontrol takes the responsibility of archiving the samples for the period of one year.

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Table of Contents

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1. <u>Summary and Conclusion:</u>

In accordance with COLIPA Guidelines for In vitro determination of SPF and PA rating of sunscreen products, test material **Ultra mineral Glo 40 + Batch No. 1772** when tested using UV 2000S Transmittance Analyzer, yielded following results-
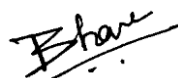
• *In-vitro SPF 43.78*

•*Critical wavelength 374.73*

•*Boots Star *****
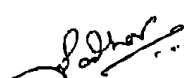
•*PA rating   PA++++*

Prepared & Analyzed by

Sign:

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Dr. Ragini Bhave
Project Manager
Clinical

Approved by

Sign:

Date: 27/12/2023
Ms. Shraddha Jadhav
General Manager
Quality Assurance

2. Aim and Objective:

To Evaluate *In-Vitro* Sun Protection Factor and PA Rating of the Sunscreen Sample.

3. Principle:

The test is based on the assessment of UV-transmittance through a thin film of sunscreen sample spread on a roughened substrate, before and after exposure to a controlled dose of radiation from a defined UV exposure source. Because of the several variables that cannot be controlled with typical thin film spectroscopic techniques, each set of sunscreen transmission data is mathematically adjusted so that the *in vitro* SPF data yield the same measured *in vivo* SPF value that was determined by *in vivo* testing.

The UV-2000S is based on the *spectral transmittance* of ultraviolet light through a sunscreen material. The term *spectral transmittance* refers to the fraction of light transmitted at a single wavelength. The UV-2000S instrument measures spectral transmittance across the 250 - 450 nm wavelength spectrum using an integrating sphere and two spectrometer instruments. The sample beam is generated inside the integrating sphere by an ultraviolet pulsed flash lamp.

4. Figure:
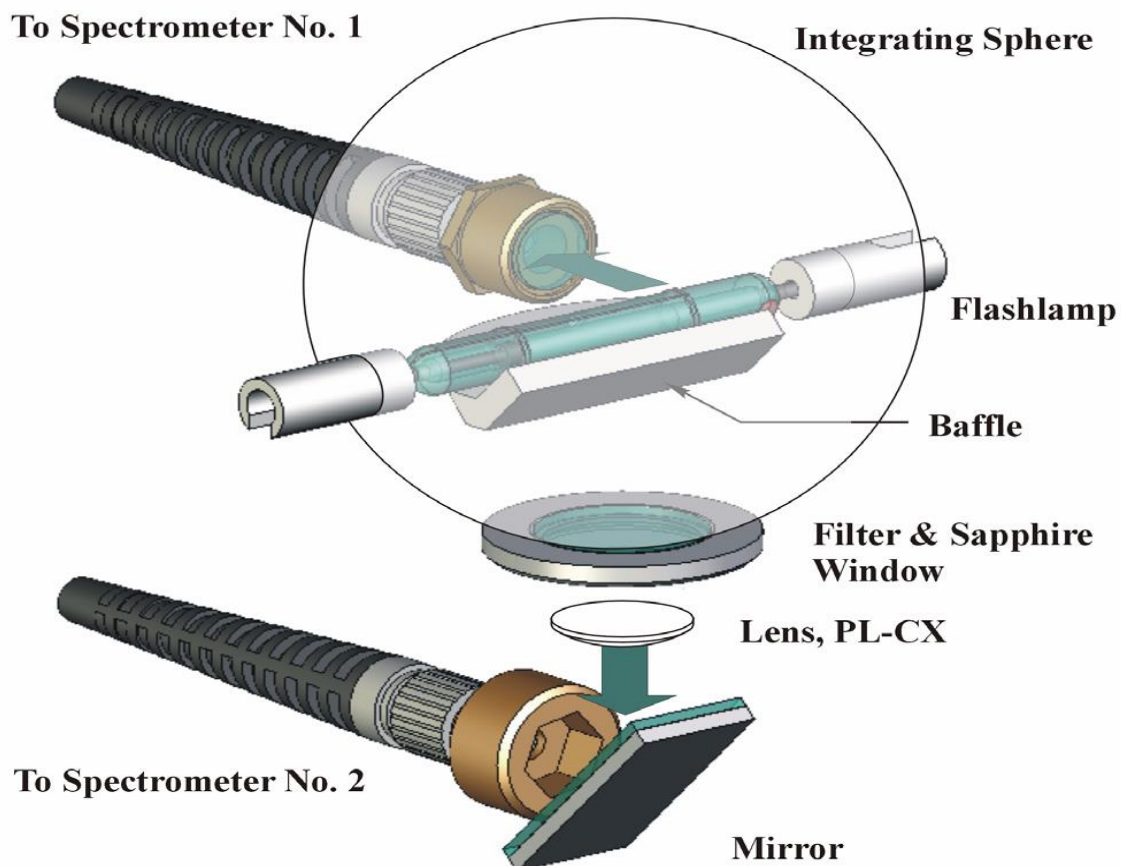
5. Materials and Methods:

5.1 Instruments:

UV 2000S Ultraviolet Transmittance analyzer, Solar Simulator, Balance.

5.2 Chemicals:

Glycerin, Distilled water

5.3 Miscellaneous:

PMMA plates, Paper towel, Pipette

6. Analysis Procedure:

6.1 Blank Plate Preparation

- Both sides of the PMMA plate were washed under clear, cold distilled water and the plate was blotted dry with a paper towel.
- The PMMA plate was placed onto the analytical balance with the roughened side up and the weight was recorded.
- Using a pipette, a number of small droplets of Glycerin were applied onto the clean plate distributed evenly over the roughened surface until the total weight of the Glycerin is 15 mg for a 50 x 50 *mm* plate.
- The plate was removed from the analytical balance and Glycerin was spread across the entire surface of the plate evenly with a finger using light strokes as quickly as possible. Stroking continued on the surface of the plate in all directions until no puddles or areas of excess agent exist. The blank plate was allowed to set aside in a dark environment for 15 minutes.
- The PMMA plate was then scanned and the transmittance results were compared with the following table.

Wavelength	Minimum Transmittance (%)
290	60
300	69
320	81

- After achieving the transmittance limit, a blank scan was taken, and the sample plate was prepared.

6.2 Sample plate preparation

- Both sides of the PMMA plate were washed under clear, cold distilled water and the plate was blotted dry with a paper towel.
- The PMMA plate was placed onto the analytical balance with the roughened side up and the weight was recorded.
- Using a pipette, several small droplets of sample were applied onto the clean plate distributed evenly over the roughened surface with the application rate of 1.3 mg/cm^2 i.e., 32.5 *mg* for a 50 x 50 *mm* plate.
- The plate was removed from the analytical balance and sample was spread across the entire surface of the plate evenly with a pre-saturated finger.
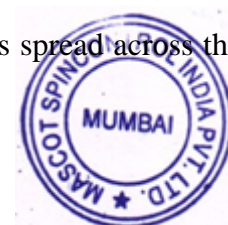
- Spreading was completed in two phase's process. First, the product was distributed over the whole area as quickly as possible *(less than 30 seconds)* using small circular motions with minimal pressure. Then the sample was rubbed on the plate surface using alternating horizontal and vertical strokes with increased moderate pressure. The second phase was completed in 20 to 30 *sec*.
- When the sample was spread evenly with no gaps, smears or excess product buildup, this treated sample was then allowed to equilibrate for at least 15 minutes in the dark at ambient temperature to help facilitate formation of a standard stabilized product film.

6.3 UV exposure using Solar Simulator (UV Source)

- Treated PMMA plates were placed on a non-reflecting surface during UV exposure.
- Total UV irradiance was done in between 50-140 Wm^{-2}
- The Sample plate was exposed into the solar simulator.

6.4 Operating Procedure

- Initially the blank plate was scanned using UV2000S transmittance Analyzer.
- Then sample plate was scanned for determination of Pre-irradiation SPF.
- The dose of UV delivered during one measurement cycle did not exceed by 0.2 J/cm^2.
- Which was followed by irradiation of the plate in solar simulator.
- Sample plate was further scanned for determination of Post-irradiation SPF.

7. Calculations:

7.1 Sunscreen Protection Factor (SPF).

SPF is a measure of the ultraviolet solar energy required to produce sunburn on skin treated with sunscreen, relative to the amount of solar energy required to produce sunburn on unprotected skin. As the SPF rating of a sunscreen product increases, sunburn protection increases. Most sunscreen manufacturers use the *in vitro* method similar to the UV light test performed by the UV-2000S to accelerate new product development, decrease in-vivo product test cycles and cost and to verify lot-to-lot manufacturing consistency. UV-2000 calculates the SPF characteristic according to the ratio.

$$SPF = \frac{\int_{290}^{400} E_\lambda \, S_\lambda d_\lambda}{\int_{290}^{400} E_\lambda \, S_\lambda T_\lambda d_\lambda}$$

Where E_λ is the erythema action spectrum, S_λ is the solar spectral irradiance, T_λ is the spectral transmittance of the sample with the integral is calculated across the 290 - 400 nm wavelength limits.

7.2 UVAPF0.

UVAPF0 is the pre-irradiation UVA Protection Factor calculated individually for each plate. First, the SPF *in vitro* sunscreen characteristic described above is adjusted to the *in vivo* SPF value determined for the same sunscreen product, to determine the coefficient of adjustment, C. UV-2000 calculates the value of the Coefficient of Adjustment C automatically and applies the coefficient to the ratio.

$$UVAPF_0 = \frac{\int_{320}^{400} P(\lambda)I(\lambda)d\lambda}{\int_{320}^{400} P(\lambda)I(\lambda)10^{-A_0(\lambda)C}d\lambda}.$$

Where P(A) is the PPD action spectrum specified in the guidelines.

The COLIPA Method recommends that C falls within a range between 0.8 and 1.2. UVAPF0 is determined before irradiating the sample and is displayed in the Plate Data Table under the heading "UVAPF Pre-irradiation".

7.3 UVAPF.

The UVAPF characteristic is computed in the same manner as the UVAPF0 calculation above, except that the calculation is made after the irradiance is applied to the sample plate. The value of the coefficient C is the same in both calculations. UV-2000 displays the UVAPF characteristic for each plate in the Plate Data Table under the heading "UVAPF".

7.4 Irradiation Dose.

The irradiation dose is calculated for each sample plate after the pre-irradiation scans are complete. The dose parameter is equal to the UVAPF0 parameter for a plate multiplied by the value 1.2 J/cm^2.

7.5 Mean UVAPF.

UV-2000 computes the average of the included plate UVAPF values and reports this characteristic at the bottom right-hand corner of the COLIPA Method Window as the "UVAPF Mean". The characteristic does not appear on the screen until sufficient number of sample plates have been tested.

UVAPF value	2 to less than 4	4 to less than 8	8 to less than 16	More than 16
Japanese PA method	PA+	PA++	PA+++	PA++++
Level of performance	Low	Medium	High	Very High

7.6 SPF: UVAPF Ratio.

The final sunscreen characteristic under the COLIPA method is the SPF: UVAPF Ratio which is the *in vivo* SPF or SPF *label* parameter divided by the UVAPF mean.

7.7 Critical Wavelength (λc).

The critical wavelength is defined across the 290 - 400 nm spectrum by the following relation:
$$\lambda_c = Min(A'),$$

such that λ satisfies the relationship:
$$\frac{\sum_{\lambda=290}^{\lambda'} A_\lambda}{\sum_{\lambda=290}^{400} A_\lambda} \geq 0.9$$

where A(λ) is the absorbance at wavelength λ.

UV-2000 displays the computed SPF and critical wavelength in the data window for the scan selected in Scan Sets Window. The mean statistics are calculated for the entire set that applies to the scan or set selected in the Scan Sets Window:

$$\text{SPF Mean} = \sum_{i=1}^{n} \frac{SPF(i)}{n}, \qquad \text{Lambda Critical Mean} = \sum_{i=1}^{n} \frac{\lambda_c(i)}{n}$$

The standard deviation statistics are calculated for the entire set that applies to the selected scan or set in the Scan Sets Window:

$$\text{SPF STD} = \sqrt{\sum_{i=1}^{n} \frac{(SPF(i) - SPF\ mean)^2}{n-1}},$$

$$\text{Lambda Critical STD} = \sqrt{\sum_{i=1}^{n} \frac{(\lambda c(i) - Lambda\ Critical\ Mean)^2}{n-1}}$$

The coefficient of variation terms are defined for each set as:

$$\text{COV} = \frac{\text{SPF}}{\text{Mean}} 100\%$$

7.8 UVA:UVB Ratio

The UVA:UVB Ratio sunscreen characteristic is calculated for pre-exposure and post-exposure conditions:

$$\text{UVA: UVB Ratio} = \frac{\text{UVA}}{\text{UVB}}$$

UV-2000 displays a UVA:UVB Ratio for each sample plate before and after the ultraviolet radiation exposure.

		INITIAL Mean UVA:UVB RATIO			
		0.0 to 0.59	*0.6 to 0.79*	*0.8 to 0.89*	*0.9 and over*
POST EXPOSURE Mean UVA:UVB RATIO	0.0 to 0.56	*No Rating*	*No Rating*	*No Rating*	*No Rating*
	0.57 to 0.75	*No Rating*	***	***	***
	0.76 to 0.85	*No Rating*	***	****	****
	0.86 and Over	*No Rating*	***	****	*****

8. **Final SPF Results:**

Parameters	SPF Mean	C Coeff	UVAPF	PA Rating	Boots Star
Plate 1	43.81	1.01	21.12	PA++++	****
Plate 2	45.66	1.00	21.19	PA++++	****
Plate 3	41.87	1.02	20.54	PA++++	****
Mean	**43.78**	**1.01**	**20.95**	**PA++++**	****

9. **Appendix:**

Appendix I

REPORT FOR *INVITRO* SUN PROTECTION FACTOR
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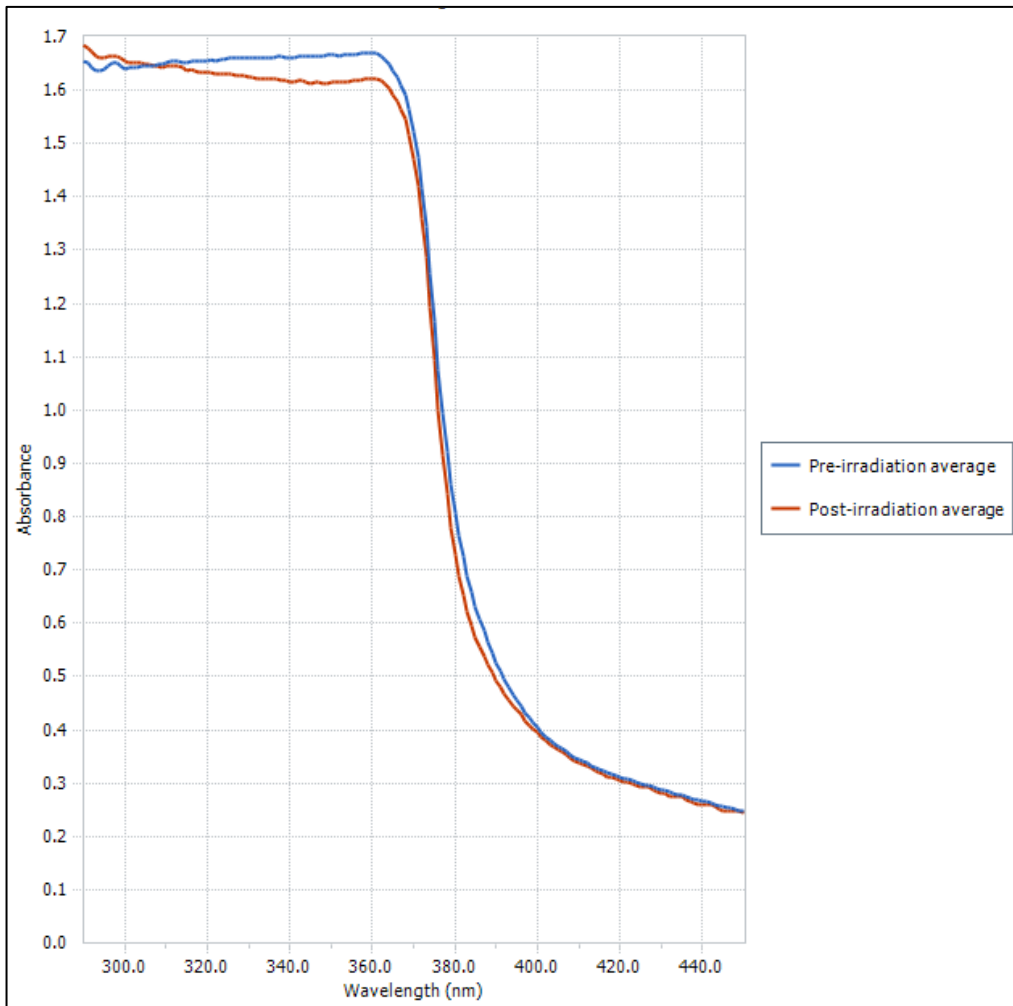
Appendix II

Pre-Irradiation Data

```
              Plate 1 pre-irradiation

                                        Lambda
                 SPF   UVAPF SPF/UVAPF UVA/UVB  Critical
Number of Scans:   5       5         5       5         5
Mean:          43.94   23.83     1.842   0.799    375.00
STD:            3.70    1.50      0.04    0.00      0.00
COV:            8.43%   6.31%     2.24%   0.05%     0.00%

              Plate 2 pre-irradiation

                                        Lambda
                 SPF   UVAPF SPF/UVAPF UVA/UVB  Critical
Number of Scans:   5       5         5       5         5
Mean:          45.69   23.90     1.912   0.800    375.00
STD:            1.71    0.67      0.02    0.00      0.00
COV:            3.75%   2.81%     1.05%   0.08%     0.00%

              Plate 3 pre-irradiation

                                        Lambda
                 SPF   UVAPF SPF/UVAPF UVA/UVB  Critical
Number of Scans:   5       5         5       5         5
Mean:          41.95   23.97     1.749   0.800    375.00
STD:            2.96    1.20      0.04    0.00      0.00
COV:            7.06%   5.01%     2.01%   0.01%     0.00%
```

Post-Irradiation Data

```
                      Plate 1 post-irradiation

                                                    Lambda
                       SPF    UVAPF SPF/UVAPF UVA/UVB Critical
Number of Scans:        5       5        5       5        5
Mean:                 43.44   21.12    2.056   0.771   374.80
STD:                   1.52    0.38     0.04    0.00     0.45
COV:                   3.51%   1.78%    1.80%   0.21%    0.12%


                      Plate 2 post-irradiation

                                                    Lambda
                       SPF    UVAPF SPF/UVAPF UVA/UVB Critical
Number of Scans:        5       5        5       5        5
Mean:                 45.15   21.20    2.129   0.772   375.00
STD:                   2.42    0.81     0.03    0.00     0.00
COV:                   5.36%   3.80%    1.56%   0.07%    0.00%


                      Plate 3 post-irradiation

                                                    Lambda
                       SPF    UVAPF SPF/UVAPF UVA/UVB Critical
Number of Scans:        5       5        5       5        5
Mean:                 40.10   20.55    1.952   0.770   374.40
STD:                   1.40    0.66     0.01    0.00     0.55
COV:                   3.50%   3.22%    0.48%   0.15%    0.15%
```

Mean Pre-irradiation Statistics

```
                          SPF        UVAPF
Number of Plates:          3            3
Mean:                    43.86       23.88
STD:                      1.87        0.08
COV:                      4.26        0.33%

UVA/UVB Ratio: 0.800
Lambda Critical: 375.00
```

Mean Post-irradiation Statistics

```
                          SPF        UVAPF
Number of Plates:          3            3
Mean:                    42.90       20.95
STD:                      2.57        0.36
COV:                      5.98        1.70%

UVA/UVB Ratio: 0.771
Lambda Critical: 374.73
```

9. References:

COLIPA Guidelines, 2011. *In vitro* method for the determination of the UVA protection factor and "critical wavelength" values of sunscreen products.